Haptoglobin polymorphism in relation to coronary plaque characteristics on radiofrequency intravascular ultrasound and near-infrared spectroscopy in patients with coronary artery disease

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ABSTRACT

Background: Conflicting results exist regarding the association between a common Haptoglobin (Hp) polymorphism and risk of coronary artery disease. We investigated the association of three functionally different anti-oxidant and anti-inflammatory Hp phenotypes (Hp1-1, Hp2-1, Hp2-2) with invasively measured degree and composition of coronary atherosclerosis as determined by intravascular ultrasound (-virtual histology) (IVUS(-VH)) as well as near-infrared spectroscopy (NIRS).

Methods: Non-culprit coronary artery segments of 581 patients with acute coronary syndrome (ACS) or stable angina pectoris were imaged with IVUS(-VH). In 203 patients, the segments were also imaged with NIRS. Pre-procedural blood samples were drawn for Hp phenotyping. Degree (segment plaque volume, segment plaque burden (PB); presence of lesions with PB ≥ 70%) and composition (segment fractions of fibrous, fibrofatty, dense calcium, and necrotic core tissue; presence of IVUS-VH derived thin-cap fibroatheroma lesions) of coronary atherosclerosis were measured.

Results: No differences were present between the three Hp phenotypes with regard to degree and composition of coronary atherosclerosis in the full cohort. However, ACS patients with a Hp2-1 or Hp2-2 phenotype had a higher segment PB percentage (β[95% CI]: 3.88[0.31–7.44], p = 0.033), increased prevalence of lesions with PB ≥ 70% (OR[95% CI]: 3.61[1.06–12.30], p = 0.040), and a tendency towards a higher segment plaque volume (β[95% CI]: 1.29[−0.04–2.62], p = 0.056) in multivariable analyses.

Conclusions: Although in the full cohort no associations could be demonstrated between Hp phenotypes and plaque characteristics, a significant association was present between phenotypes resulting from a genotype containing a Hp2 allele (Hp2-1 or Hp2-2) and a higher degree of atherosclerosis in patients with ACS.

1. INTRODUCTION

Circulating haptoglobin (Hp) is hypothesized to influence atherosclerosis through its anti-oxidant and immunomodulatory properties. Specifically, it prevents hemoglobin-driven oxidative reactions in response to intraplaque hemorrhage, and stimulates a variety of pro- and anti-inflammatory cytokines [1,2]. The Hp gene carries a common polymorphism with two alleles (Hp1 and Hp2), resulting in three functionally-different phenotypes, each characterized by a unique protein structure: Hp1-1 (wildtype genotype; dimer), Hp2-1 (heterozygous variant; linear polymer) and Hp2-2 (homozygous variant; cyclic polymer) [2]. The homozygous variant Hp2-2 produces a dysfunctional protein with the lowest anti-oxidant and anti-inflammatory properties as compared to the proteins encoded by Hp2-1 or Hp1-1 [1,2].

Although the molecular functions of these proteins in the vascular wall have been well investigated and seem to be clear, clinical studies on the association of Hp phenotypes with coronary events have rendered conflicting results [3,4]. In order to further increase understanding of the pathophysiological relation between Hp phenotypes and coronary atherosclerosis, imaging studies using coronary angiography and CT angiography have been performed. However, these have not been able to further elucidate potential mechanisms [5,6]. The imaging techniques used in these studies only enable evaluation of the lumen of the coronary artery. Conversely, radiofrequency intravascular ultrasound (IVUS) and near-infrared spectroscopy (NIRS) enable evaluation and quantification of the arterial wall itself. However, studies on Hp phenotype and invasively-measured coronary atherosclerotic plaque characteristics by IVUS or NIRS are currently lacking.

In the current study, we investigated the relation between Hp phenotype and in-vivo measurements of degree and composition of coronary atherosclerosis by IVUS and NIRS in 581 patients undergoing coronary angiography. Herewith, we aimed to provide additional insights into the pathophysiology concerning Hp and coronary atherosclerosis.

2. METHODS

The rationale and design of the ATHEROREMO-IVUS study and its ATHEROREMO-NIRS substudy have been described in detail elsewhere [7–9]. These studies were approved by the medical ethics committee of the Erasmus MC and performed in accordance with the declaration of Helsinki. All included patients provided written informed consent.

In brief, 581 patients with an indication for coronary angiography due to stable angina pectoris (SAP) or acute coronary syndrome (ACS) underwent IVUS imaging of a nonstenotic segment of at least 40 mm in length in a predefined non-culprit coronary artery with the Volcano™ s5/s5i Imaging System (Volcano Corp., San Diego, USA), using the

Volcano™ Eagle Eye Gold IVUS catheter (20MHz) [7]. The order of preference for selection of the non-culprit coronary artery segment was: 1. Left anterior descending artery; 2. Right coronary artery; and 3. Left circumflex artery. An automatic pullback system was used with a standard pull back speed of 0.5mm per second. Both IVUS grayscale and virtual histology (IVUS-VH) analyses were performed using pcVH 2.1 and qVH (Volcano Corp., San Diego, USA) software. The external elastic membrane and luminal borders were contoured for each frame (median interslice distance, 0.40mm). The degree and composition of each atherosclerotic plaque were assessed. Plaque volume (mm^3) was defined as the total volume of the external elastic membrane occupied by atheroma and normalized for the length of the imaged segment. Plaque burden (%) was defined as plaque and media cross-sectional area divided by external elastic membrane cross-sectional area and is presented as a percentage. Atherosclerotic plaque composition was characterized into fibrous (FI), fibro-fatty (FF), dense calcium (DC) and necrotic core (NC) tissue and expressed as percentages of total plaque volume. Three types of high-risk lesions were identified: 1. Virtual Histology-IVUS derived thin-cap fibroatheroma (VH-TCFA) lesions (presence of N10% confluent necrotic core in direct contact with the lumen); 2. Lesions with plaque burden ≥70%; and 3. Lesions with a minimal luminal area ≤4.0mm^2. Hp phenotypes were successfully determined in 574 of the patients who underwent IVUS(-VH) imaging (Fig. 1). NIRS (InfraReDx, Burlington, Massachusetts, USA) of the same segment was performed in a subset of 191 patients, as well as 12 additional patients that only underwent NIRS, not IVUS [7,9] (Fig. 1). The U.S. Food and Drug

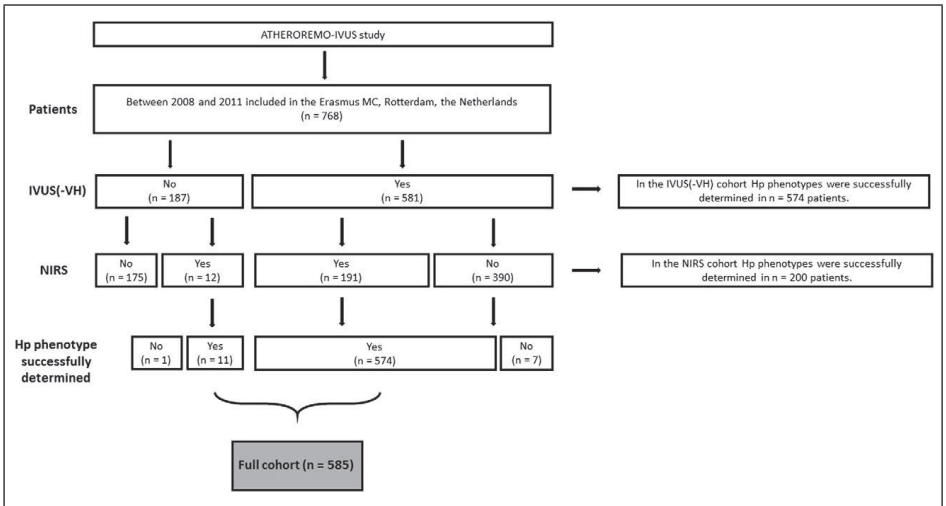

Fig. 1. Flowchart patient inclusion in the ATHEROREMO-IVUS study, ATHEROREMO-NIRS substudy and ATHEROREMO Haptoglobin phenotype substudy.
ACS=acute coronary syndrome; Hp = haptoglobin; IVUS(-VH) = intravascular ultrasound(-virtual histology); NIRS = near-infrared spectroscopy.

Administration-approved NIRS system, as used in this study, consisted of a 3.2-F rapid exchange catheter, a pullback and rotation device. Image acquisition was performed by a motorized catheter pullback at a speed of 0.5 mm per second and 240 rotations per minute. The Lipid Core Burden Index (LCBI) score was measured and represents the amount of lipid core in the imaged segment on a 0-to-1000 scale, as described previously [9]. Both IVUS and NIRS images were analyzed offline by an independent core laboratory (Cardialysis BV, Rotterdam, the Netherlands), by personnel blinded for baseline patient characteristics and Hp phenotypes. Two-hundred of the 203 patients who underwent NIRS imaging, were successfully phenotyped, resulting in a total of 585 successfully phenotyped and imaged patients for the current investigation (Fig. 1).

EDTA plasma samples were drawn from the arterial sheath prior to coronary angiography and transported to the clinical laboratory of the Erasmus MC for further processing and storage at a temperature of −80°C within 2 h after blood collection. After completion of the cohort, all frozen EDTA-plasma samples were transported under controlled conditions (at a temperature of −80°C) to the Laboratory of Experimental Cardiology, University Medical Center Utrecht, Utrecht, The Netherlands, where Hp phenotypes were differentiated through western blotting.

The primary endpoint consisted of major adverse cardiovascular events (MACE), and was defined as a composite of all-cause mortality, ACS, and unplanned coronary revascularization. The secondary endpoint consisted of the composite of all-cause mortality and ACS. ACS was defined as the clinical diagnosis of (non-)ST-segment elevation myocardial infarction or unstable angina pectoris [10,11]. Unplanned coronary revascularization was defined as any repeat PCI or coronary artery bypass grafting that was not foreseen at the index procedure. Follow-up data were collected during 1 year. Vital status was obtained from municipal civil registries and questionnaires were sent focusing on the occurrence of MACE to all living patients (response rate of 92.3%). Upon patients' approval, additional information was obtained from hospital discharge letters and treating physicians whenever necessary. Endpoints were adjudicated based on original source data by a clinical events committee.

Variables with a non-normal distribution were transformed by using either the natural logarithm (ln) or square root for further analyses. Univariate analyses were performed by ANOVA or Student's t-test for continuous variables and Chi-squared test for categorical variables, comparing the phenotypes. Multivariate linear and logistic regression analyses were performed with Hp phenotype as the independent variable and with adjustment for the potential confounders age, gender, smoking, diabetes mellitus, hypertension, dyslipidemia and previous myocardial infarction. Interaction terms were used to test for effect modification by indication for angiography (ACS versus SAP). Subsequently, analyses were stratified on indication. Since ACS subgroup analysis showed (VH-)IVUS values of similar magnitude in the Hp2-1 and Hp2-2 groups, a post-hoc analysis was

performed comparing ACS patients with wildtype Hp1-1 versus a pooled group with the variant phenotypes Hp2-1 and Hp2-2. Furthermore, a subgroup analysis was performed in diabetic patients (n = 99). Finally, the association between Hp phenotype and clinical endpoints after 1 year of follow-up was examined with Cox proportional hazard regression analyses.

All data were analyzed with SPSS software (IBMSPSS Statistics for Windows, Version 23, Armonk, NY, USA). All statistical tests were two-tailed and p-values < 0.05 were considered statistically significant.

3. RESULTS

3.1. Baseline characteristics

Baseline clinical and procedural characteristics of the 3 phenotype groups are presented in Table 1. Prevalence of phenotype Hp1-1, Hp2- 1 and Hp2-2 was 16.1% (n = 94), 45.5% (n = 266), and 38.5% (n = 225), respectively, and this distribution was in Hardy–Weinberg equilibrium (p = 0.67). Mean age ± standard deviation was 61.9 ± 12.6, 62.4 ± 10.7 and 60.0 ± 11.4 years, respectively (p = 0.07). Except for history of myocardial infarction, there were no differences in clinical or procedural characteristics across the various phenotypes. As expected, circulating plasma haptoglobin concentration was lowest for Hp1-1 and highest for Hp2-2 (p=<0.001, Table 1).

3.2. Degree and composition of coronary atherosclerosis

No differences could be demonstrated between the different phenotypes with regard to the degree and composition of atherosclerosis as assessed by IVUS-VH or NIRS (Table 2). The same could be concluded for the subgroup analysis of diabetic patients (n=99, data not shown).

Significant interactions were present between Hp phenotype and indication for angiography (ACS versus SAP) for the association with plaque volume, plaque burden, FI tissue percentage and lesions with PB ≥ 70% (p-values for interaction all <0.05 in uni- and multivariate analysis). In line with this, in ACS patients, phenotypes resulting from a genotype containing a Hp2 allele (Hp2-1 or Hp2-2) were significantly associated with a higher plaque volume (p=0.031) in univariate analysis and tended to be associated with a higher plaque volume in multivariate analysis (β [95% CI]: 1.29 [−0.04–2.62] mm3 increase in (square root transformed) plaque volume for having Hp2-1 or Hp2-2 as compared to Hp1-1, p = 0.056) (Table 3, Fig. 2). Moreover, in ACS patients these phenotypes were independently associated with a larger plaque burden (β [95% CI]: 3.88 [0.31–7.44]% increase in PB for having Hp2-1 or Hp2-2 as compared to Hp1-1, p = 0.033) (Table 3, Fig. 3), as well as an increased prevalence of lesions with PB ≥ 70%

Table 1. Baseline clinical and procedural characteristics of the haptoglobin phenotype groups in the full cohort (n= 585).

Clinical characteristics	Haptoglobin 1-1 (n = 94)	Haptoglobin 1-2 (n = 266)	Haptoglobin 2-2 (n = 225)	P-value[a]
Age, years	61.9 ± 12.6	62.4 ± 10.7	60.0 ± 11.4	0.07
Male gender, n (%)	69 (73.4)	200 (75.2)	173 (76.9)	0.79
Diabetes mellitus, n (%)	11 (11.7)	46 (17.3)	43 (19.1)	0.28
Hypertension, n (%)	48 (51.1)	138 (51.9)	118 (52.7)	0.96
Dyslipidemia, n (%)	45 (47.9)	154 (57.9)	126 (56.3)	0.24
Smoking, n (%)	25 (26.6)	83 (31.2)	63 (28.0)	0.61
Positive family history, n (%)	58 (61.7)	139 (52.3)	110 (49.1)	0.12
Peripheral artery disease, n (%)	4 (4.3)	18 (6.8)	14 (6.2)	0.68
Previous myocardial infarction, n (%)	24 (25.5)	98 (36.8)	64 (28.4)	0.050
Previous PCI, n (%)	26 (27.7)	91 (34.2)	73 (32.4)	0.51
Previous CABG, n (%)	3 (3.2)	9 (3.4)	7 (3.1)	0.99
Previous stroke, n(%)	3 (3.2)	12 (4.5)	10 (4.4)	0.85
History of renal insufficiency, n (%)	4 (4.3)	21 (7.9)	8 (3.6)	0.10
Haptoglobin level, mg/ml	0.79 [0.58–0.99]	1.53 [1.10–2.20]	1.60 [1.10–2.30]	<0.001
Procedural characteristics				
Indication for coronary angiography				
Acute coronary syndrome, n (%)	49 (52.1)	144 (54.1)	127 (56.4)	0.76
Stable angina pectoris, n (%)	45 (47.9)	122 (45.9)	98 (43.6)	0.76
Coronary artery disease				
No significant stenosis, n (%)	4 (4.3)	17 (6.4)	22 (9.8)	0.16
1-vessel disease, n (%)	57 (60.6)	138 (51.9)	117 (52.0)	0.30
2-vessel disease, n (%)	26 (27.7)	82 (30.8)	60 (26.7)	0.58
3-vessel disease, n (%)	7 (7.4)	29 (10.9)	26 (11.6)	0.54
PCI performed	86 (91.5)	233 (87.6)	195 (86.7)	0.48

Continuous variables are presented as mean ± SD or median [interquartile range], depending on their distribution. Categorical variables are presented as n (%).
PCI= percutaneous coronary intervention; CABG= coronary artery bypass graft surgery.
[a] P-values obtained by ANOVA for the continuous variables and Chi-squared test for the categorical variables.

(OR[95% CI]: 3.61 [1.06–12.30], p = 0.040) (Table 3, Fig. 4). With respect to atherosclerotic plaque composition, no associations were present with the various VH-tissue types, LCBI or VH-TCFA lesions in ACS patients (Table 3).

Table 2. NIRS and (VH-)IVUS segment and lesion characteristics of the Haptoglobin phenotype groups in the full cohort (n = 585).

	Haptoglobin 1-1 (n = 94)	Haptoglobin 1-2 (n = 266)	Haptoglobin 2-2 (n = 225)	P-value[c]
Segment plaque characteristics[a]				
Degree of atherosclerosis				
Plaque volume, mm^3	240.7 [118.5–313.4]	235.1 [150.8–332.9]	216.0 [147.6–323.3]	0.94
Plaque burden, %	37.4 ± 12.1	38.3 ± 11.6	38.4 ± 11.1	0.80
Plaque composition				
Fibrous percentage	57.6 ± 12.0	57.4 ± 11.4	58.2 ± 11.7	0.74
Fibro-fatty percentage	9.1 [5.9–12.4]	9.2 [5.9–13.3]	8.4 [5.3–11.9]	0.19
Necrotic core percentage	21.7 ± 8.1	21.0 ± 8.3	21.8 ± 7.7	0.57
Dense calcium percentage	9.5 [5.3–14.4]	9.5 [5.4–15.3]	9.1 [4.9–15.1]	0.92
Lipid Core Burden Index (LCBI)[b]	47.5 [9.0–93.5]	40.5 [16.0–85.8]	40.0 [13.3–80.8]	0.82
Lesion plaque characteristics[a]				
Degree of atherosclerosis				
≥1 Lesion with PB ≥70%, n (%)	18 (20.2)	54 (20.5)	51 (23.2)	0.74
≥1 Lesion with MLA ≤4.0 mm^2, n (%)	29 (32.6)	83 (31.7)	68 (30.9)	0.96
Plaque composition				
≥1 TCFA, n (%)	43 (48.3)	106 (40.3)	91 (41.2)	0.40

Continuous variables are presented as mean ± SD or median [interquartile range], depending on their distribution. Categorical variables are presented as n (%).
PB= plaque burden; MLA = minimal lumen area; and TCFA = thin-cap fibroatheroma.
[a] IVUS(-VH) imaging was performed in 574 patients.
[b] NIRS imaging was performed in a subset of 200 patients.
[c] P-values obtained by ANOVA for the continuous variables and Chi-squared test for the categorical variables.

3.3. Clinical endpoints

With regard to clinical outcome, associations between Hp phenotype and 1-year cardiovascular outcome could not be demonstrated, both in the full cohort and in the ACS and diabetes subgroups. In particular, the Hp phenotypes were not associated with the occurrence of MACE (primary composite endpoint) on multivariate analysis: HR[95% CI] 0.88 [0.52–1.49] for Hp2-1 and 0.97 [0.57–1.67] for Hp2-2 in the full cohort; HR[95% CI] 0.77 [0.38–1.56] for Hp2-1 and 0.70 [0.33– 1.49] for Hp2-2 in the ACS subgroup; HR [95% CI] 0.91 [0.30–2.82] for Hp2-1 and 0.94 [0.30–3.02] for Hp2-2 in the diabetic subgroup.

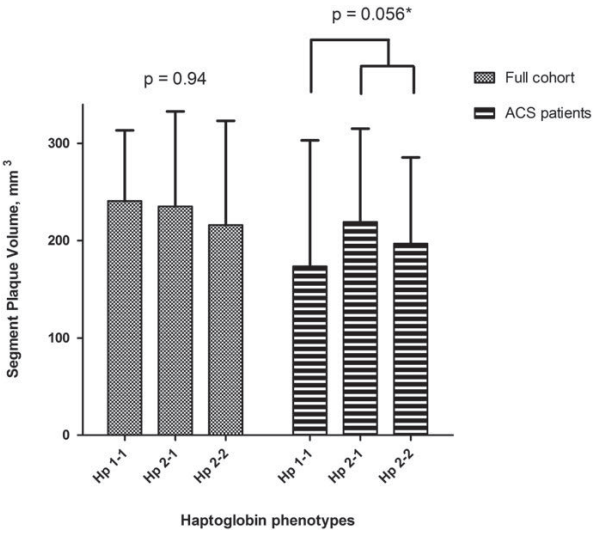

Fig. 2. Segment plaque volume in the haptoglobin phenotypes within the full cohort and within the ACS subgroup.

* P-value for difference in segment plaque volume between Hp2-1 or Hp2-2 as compared to Hp1-1 within the ACS subgroup. Adjusted for age, gender, smoking, diabetes mellitus, hypertension, dyslipidemia and previous myocardial infarction. ACS = acute coronary syndrome; and Hp = haptoglobin.

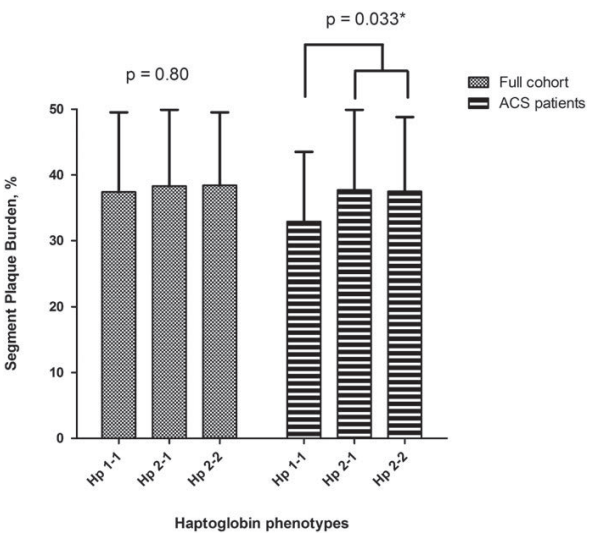

Fig. 3. Segment plaque burden in the haptoglobin phenotypes within the full cohort and ACS subgroup.

*P-value for difference in segment plaque burden between Hp2-1 or Hp2-2 as compared to Hp1-1 within the ACS subgroup. Adjusted for age, gender, smoking, diabetes mellitus, hypertension, dyslipidemia and previous myocardial infarction. ACS = acute coronary syndrome; Hp= haptoglobin.

Table 3. NIRS and (VH)-IVUS segment and lesion characteristics of the haptoglobin phenotype groups in patients with ACS (n = 320).

	Haptoglobin 1-1 (n = 49)	Haptoglobin 2-1 or 2-2 (n = 271)	P-value[c]	β [95% CI]	P-value[d]
Haptoglobin level, mg/ml	0.84 [0.63–1.10]	1.60 [1.10–2.50]	<0.001	0.65 [0.45–0.85]	<0.001
Segment plaque characteristics[a]					
Degree of atherosclerosis					
Plaque volume, mm^3	173.5 [107.3–303.1]	215.5 [141.2–304.2]	0.031	1.29 [–0.04–2.62]	0.056
Plaque burden, %	32.9 ± 10.6	37.6 ± 11.8	0.014	3.88 [0.31–7.44]	0.033
Plaque composition					
Fibrous percentage	61.4 ± 11.2	58.5 ± 12.0	0.13	–2.99 [–6.80–0.83]	0.12
Fibro-fatty percentage	8.6 [4.6–12.0]	8.6 [5.5–12.0]	0.35	0.18 [–0.14–0.51]	0.26
Necrotic core percentage	21.2 ± 8.3	21.8 ± 8.6	0.68	0.43 [–2.37–3.22]	0.76
Dense calcium percentage	6.7 [4.9–11.3]	8.3 [4.9–13.8]	0.28	0.21 [–0.17–0.58]	0.28
Lipid Core Burden Index (LCBI)[b]	48.0 [6.0–91.0]	44.5 [16.0–88.0]	0.53	0.03 [–0.71–0.77]	0.93
Lesion plaque characteristics[a]			P-value[c]	OR [95% CI]	P-value[d]
Degree of atherosclerosis					
≥1 Lesion with PB ≥ 70%, n (%)	3 (6.7)	56 (21.0)	0.023	3.61 [1.06–12.30]	0.040
≥1 Lesion with MLA ≤4.0 mm^2, n (%)	11 (24.4)	80 (30.1)	0.44	1.30 [0.61–2.7	0.51
Plaque composition					
≥1 TCFA, n (%)	22 (48.9)	119 (44.6)	0.59	0.78 [0.41–1.51]	0.46

Continuous variables are presented as mean ± SD or median [interquartile range], depending on the distribution. Categorical variables are presented as n (%).

Beta (β) indicates the increase or decrease (minus sign) in each (transformed) imaging segment parameter for the Haptoglobin 2-1 or 2-2 ACS patients as compared to Haptoglobin 1-1 ACS patients.

Odds ratio (OR) increase in each lesion parameter for the Haptoglobin 2-1 or 2-2 ACS patients as compared to Haptoglobin 1-1 ACS patients.

[a] IVUS-VH imaging was performed in 313 ACS patients.

[b] NIRS imaging was performed in a subset of 93 ACS patients.

[c] P-values (univariate) obtained by the independent Student's two-sample t-test for the continuous variables and Chi-squared test for the categorical variables.

[d] P-values (multivariate) obtained by linear regression analyses for continuous variables and logistic regression analyses for categorical variableswith Haptoglobin 1-1 as the reference category. Models adjusted for age, gender, smoking, diabetes mellitus, hypertension, dyslipidemia and previous myocardial infarction.

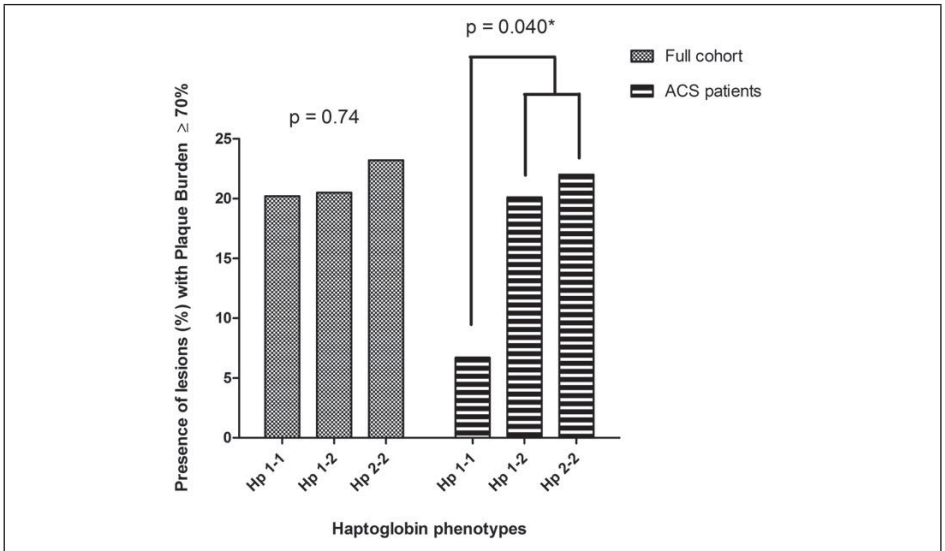

Fig. 4. Presence of large lesions in the haptoglobin phenotypes within the full cohort and ACS subgroup.

*P-value for difference in the presence of large lesions between Hp2-1 or Hp2-2 as compared to Hp1-1 within the ACS subgroup. Adjusted for age, gender, smoking, diabetes mellitus, hypertension, dyslipidemia and previous myocardial infarction. ACS = acute coronary syndrome; Hp= haptoglobin.

4. DISCUSSION

To our knowledge, this is the first study that investigated the relation between Hp phenotypes and coronary plaque characteristics as assessed with IVUS-VH and NIRS in patients with CAD. Although no associations could be demonstrated between Hp phenotypes and coronary plaque characteristics in the full cohort, in ACS patients phenotypes Hp2-1 and Hp2-2 were significantly associated with a higher degree of coronary atherosclerosis as expressed by higher segment plaque burden and higher prevalence of lesions with PB ≥ 70%, as compared to Hp1-1.

Existing imaging studies on Hp phenotype and atherosclerosis as assessed with either coronary angiography or CT angiography are limited in number and were mainly performed in specifically defined study populations, such as patients with diabetes mellitus [5,6,12,13]. Overall, these studies did not find any associations between Hp phenotype and coronary atherosclerosis [5,6,12,13]. The interaction between Hp phenotype and acute versus stable clinical presentation of CAD has not been investigated earlier. A potential, biologically plausible explanation for this interaction could be that patients who ultimately experience ACS represent a subgroup that exhibits an increased pro-inflammatory [14] and oxidative state [15–17] as compared to SAP patients. In these patients, elevated oxidative stress may not only be present systemically [15,16] but also

locally at the level of the atherosclerotic plaque, as part of the pathogenesis and evolution towards an ACS. The latter results, among others, from intraplaque hemorrhage, which occurs more often in ACS than in SAP patients, and gives rise to a local release of hemoglobin (iron) into the atherosclerotic plaque [18]. Such a state leads, among several other reactions, to local generation of reactive oxygen species and consequently lipid peroxidation [15], which eventually may contribute to accelerated atherosclerotic plaque growth [15,19] in these patients. This process may be further enhanced in Hp2 phenotypes (Hp2-1 and Hp2-2) due to their reduced anti-oxidant and anti-inflammatory properties as compared to Hp1-1 [1,2]. A previous study in mice supports this hypothesis by demonstrating increased iron, lipid peroxidation and macrophage accumulation in Hp2-2 atherosclerotic plaques as compared with Hp1-1 plaques [20]. This was confirmed in humans by autopsy studies that have demonstrated more advanced atherosclerotic plaques in Hp2-2 compared to Hp1-1 individuals [21]. In contrast to Hp1-1 proteins, the Hp2-1 and Hp 2-2 proteins have low affinity for both hemoglobin and the macrophage CD163 scavenger receptor in order to clear hemoglobin (iron) from the atherosclerotic plaque and prevent its harmful intraplaque oxidative reactions [2,22]. Altogether, these studies indicate that oxidative stress might strongly be implicated in the atherosclerotic process with a critical role for Hp proteins in its further development.

In a previous study within the same cohort, we could not demonstrate an association between plasma Hp concentration and (VH-)IVUS plaque characteristics or clinical events [8]. Although the biological function of Hp in the vascular wall might not directly depend on its plasma concentrations, but rather on its protein structure, there is a direct correlation of Hp phenotype with Hp plasma concentrations. Specifically, Hp concentration is higher in Hp2-2 than in Hp1-1 individuals, because of the weaker binding of Hp2-2 proteins to hemoglobin and the macrophage CD163 receptor [22]. Thus, since Hp concentrations may at least in part be phenotype-dependent, these negative results seem consistent with our current findings.

Epidemiologic studies investigating the association between Hp phenotype and incidence of CAD in the general population are limited in number and have yielded contradicting results. While De Baquer et al. found that Hp1-1 individuals were at higher risk of CAD mortality as compared to the other Hp phenotypes [3], the Framingham Heart Offspring Study (n=3273) could not demonstrate any relationship between Hp phenotype and CAD prevalence in the overall study population [4].

The majority of clinical studies concerning Hp phenotypes has focused on diabetic individuals, since strong evidence exists that Hp phenotype and diabetic state significantly interact with regard to prevalence of CAD. It has been demonstrated that Hp2-2 individuals with diabetes have a higher risk of adverse cardiovascular outcomes as compared to the other phenotypes [4,23–27], which is thought to be caused by the decreased anti-oxidant capabilities of the Hp2-2 protein in conjunction with an exception-

ally high level of oxidative stress in diabetes [28,29]. However, some other studies could not confirm these findings [30], or even rendered contradictory results [4]. We also could not demonstrate any associations with in-vivo coronary plaque characteristics or 1-year clinical outcome in diabetic patients. Our findings are in agreement with a study in type 2 diabetic patients, that could not demonstrate an association between Hp genotype and coronary artery calcification (CAC) as a reflection of total coronary atherosclerotic burden [5]. On the other hand, a larger case-control study on type 1 diabetic patients found that the Hp2-2 genotype was a predictor of CAC progression. The limited number of diabetic participants (n = 99) in our cohort may have contributed to the lack of such an association in our study.

Our study has several limitations that warrant acknowledgement. Firstly, our findings in the ACS subgroup should be considered as hypothesis-generating, because the comparison of Hp2-2 and Hp2-1 on the one hand with Hp1-1 on the other hand in the ACS patients was a post-hoc analysis. Nevertheless, the interaction terms between Hp phenotypes and indication for catheterization were highly significant in multivariate analyses. Secondly, IVUS(-VH) imaging took place of a non-culprit coronary artery segment only. However, this approach was developed under the hypothesis that such a non-culprit target segment adequately reflects coronary wall pathophysiology of the larger coronary tree, and this hypothesis has been confirmed by several studies [31,32]. Finally, this study was not primarily designed to investigate the association between Hp phenotypes and atherosclerosis and clinical outcome in diabetic patients. A small number of diabetic patients in this study may have contributed to the lack of significant associations between Hp phenotypes and degree and composition of atherosclerosis in this subgroup.

In conclusion, in patients undergoing coronary angiography, no associations were present between Hp phenotypes and invasively measured coronary atherosclerotic plaque characteristics by IVUS and NIRS. However, patients with Hp2-1 or Hp2-2 presenting with ACS had a significantly higher degree of coronary atherosclerosis as compared to Hp1-1. Thus, genetic differences in the endogenous antioxidant status, as reflected by the haptoglobin phenotype, may be of considerable importance in patients suffering from CAD. Our hypothesis-generating findings should be confirmed by other, large studies in order to identify patient groups that might benefit from risk stratification by Hp phenotyping in the future.

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Conflict of interest

None.

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